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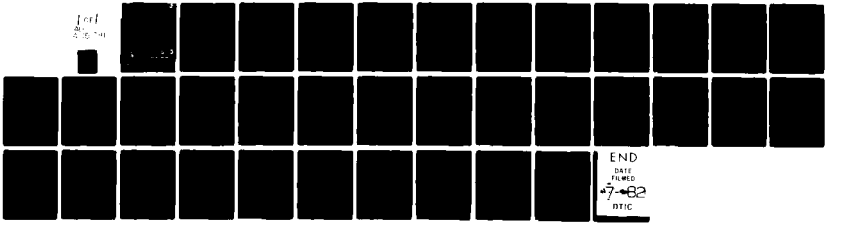

BIOLOGY OF ALKYLPHOSPHONIC ACIDS

(A Review of the Distribution, Metabolism, and Structure of Naturally Occurring Alkylphosphonic Acids)

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BIOLOGY OF ALKYLPHOSPHONIC ACIDS*

A Review of the Distribution, Metabolism, and Structure of Naturally Occuring Alkylphosphonic Acids

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BIOLOGY OF ALKYLPHOSPHONIC ACIDS

Alkylphosphonic acids, compounds having a covalent carbon-phosphorus bond as a unique characteristic, were first synthesized chemically in 1946 (Finkelstein, 1946) and discovered as naturally occurring components of biological molecules in 1959 (Horiguchi and Kandatsu, 1959). Since that time, phosphonate-phosphorus, predominantly in the form of 2-aminoethylphosphonic acid (AEP, $NH_2CH_2CH_2PO_3H_2$), has been identified from diverse species of microorganisms, marine invertebrates, and mammals. Several reviews have appeared (Horiguchi, 1966; Quin, 1967; Kittredge and Roberts, 1969; Mastalerz, 1969; Horiguchi, 1972b; Rosenberg, 1973; Engel, 1977) which cover the chemical properties and known distribution of phosphonates.

I. Properties and Identification of AEP

The first synthesis of AEP was performed by Finkelstein (Finkelstein, 1946). Since then AEP has been synthesized by a variety of methods (Kosolapoff, 1947; Chavane, 1947; Barycki et al., 1971), all of which share a step in which a phosphorus acid derivative is alkylated to form the direct carbon to phosphorus bond (C-P). Figure 1 illustrates the alkylation step from the synthesis procedure of Kosolapoff.

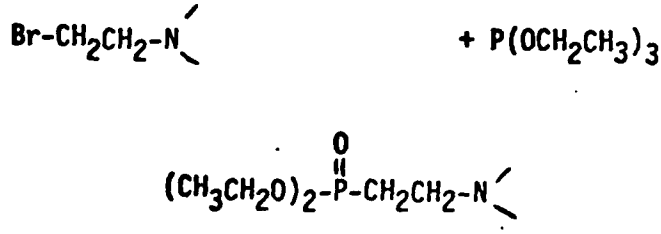


Fig. 1

Phosphonate-phosphorus has been taken as the difference between phosphorus released by acid hydrolysis and the total phosphorus content. Hydrolyzable phosphorus can be determined colorimetrically (Chen et al., 1956) following hydrolysis of the sample in 6 N HCl; however, determination of total phosphorus requires combustion (Quin, 1964) or ashing followed by a colorimetric phosphorus determination (Chen et al., 1956; Kirkpatrick and Bishop, 1971b). Snyder and Law have developed a procedure using enzymatic and chemical hydrolysis for determination of hydrolyzable phosphorus (Snyder and Law, 1970).

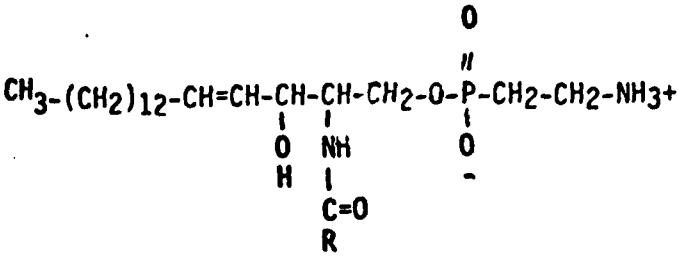
Fluorescence reactions of aminophosphonic acids (including AEP) have been investigated recently for use in quantitative determinations (Fourche et al., 1976). O-Diacetylbenzene was found to be useful for determinations of AEP at excitation and emission wavelengths of 355 and 445 nm, respectively. In early studies, AEP was quantitated by isolation from hydrolysates of whole animals or fractions of whole animals. AEP was eluted from Dowex 1 (acetate form) using 0.5 N acetic acid (Horiguchi and Kandatsu, 1959; Quin, 1964). The AEP can be recrystallized from water by addition of ethanol and the purity determined by paper chomatography. AEP has also been purified using Amberlite IR-120, Amberlite CG-120, and Dowex 1 (Shimizu et al., 1965). AEP reacts at pH 9 with fluorodinitrobenzene; following adjustment of the reaction mixture to pH 1, the DNP-AEP can be extracted with ethyl acetate (Quin, 1967). The yellow DNP derivative can be compared visually with a standard DNP-AEP after thin layer chromatography.

^{31}P NMR was proposed for the direct determination of phosphonate-phosphorus (Quin, 1965) and then applied to the detection of biological phosphonates when instrumentation with increased sensitivity became available (Glonek et al., 1970; Henderson et al., 1971). A method using Fourier Transform ^{31}P NMR has been developed for the detection of phosphonate-phosphorus in biological macromolecular material (Hilderbrand et al., 1971). Recently,

gas-liquid chromatography and mass spectrometry (GC-MS) have been used in the detection and characterization of various forms of AEP. Volatile derivatives were obtained by sequential acetylation and methylation (Alhadeff and Daves, 1970), or by trimethylsilylation (Fource et al., 1968; Karlsson, 1970). AEP has a pK_1, pK_2, and pK_3 of 2.45, 7.00, and 10.8, respectively. Polymorphism has been demonstrated for AEP and two crystalline forms are known. Although AEP has a melting point above 280°C, the melting point is not reliable for identification because the different forms melt over a temperature range of about 20° (Quin, 1967). Stability constants have been determined for AEP and a number of divalent metal ions (Sakurai et al., 1976).

II. Distribution of Phosphonates in Nature

AEP was first proposed as a possible biological molecule by Chavane in 1947 (Chavane, 1947; Chavane, 1949). The natural occurrence of phosphonates was demonstrated in 1959 when Horiguchi and Kandatsu isolated AEP from an hydrolyzed ether-ethanol extract of sheep rumen protozoa (Horiguchi and Kandatsu, 1959). AEP was initially identified via paper chromatography as a ninhydrin positive phosphate-containing spot. Without knowledge of the work of Horiguchi and Kandatsu, Kittredge et al. isolated and identified free AEP and a glycerol ester of AEP from the anemone Anthopleura elegantissima , and noted that hydrolysis of a crude lipid extract released significant amounts of AEP (Kittredge et al., 1962). The latter observation eventually led to the discovery of AEP in glycerophospholipids and to the identification of a ceramide aminoethylphosphonate (Rouser et al., 1963; Simon and Rouser, 1967).



Ceramide Aminoethylphosphonate

Detailed characterization using GC-MS identified different bases in the sea anemone Metridium senile and the oyster Ostrea gigas (hexadecasphinga-4, 8-dienine and hexadecasphinga-4-enine, respectively) while hexadecanoic acid was identified as the major fatty acid in both species (Matsubara and Hayashi, 1973; Karlsson and Samuelsson, 1974; Matsubara, 1975).

The alkylphosphonic acid, AEP, has been isolated in a free (unbound) form, a protein form, and a lipid form in the protozoa Tetrahymena pyriformis (Rosenberg, 1964). Although seventy-seven percent of the phospholipids of the cilia membrane contain AEP (Smith et al., 1970) this lipid fraction decreases during replacement of tetrahymanol with ergosterol (Nozawa et al., 1975). One of the lipid-bound forms of AEP in T. pyriformis was identified as diacylglycerol-AEP (Sugita and Hori, 1971). Other lipid-bound forms isolated from T. pyriformis include ceramide-AEP (Carter and Gaver, 1967), plasmalogen-AEP (Dawson and Kemp, 1967), and the alkoxyacylglycerol ester of AEP (Smith and Law, 1970b).

It was found that sea anemones contain AEP in the free form, bound to lipid as the glycerol ester (Kittredge et al., 1962), bound to lipid as ceramide-AEP (Mason, 1972), and in species of the genus Metridium, bound to glycoprotein (Kirkpatrick and Bishop, 1971; Kirkpatrick and Bishop, 1972; Kirkpatrick and Bishop, 1973; Hilderbrand et al., 1971; Hilderbrand et al.,

1973). The N-methyl derivatives of AEP have also been isolated from sea anemones (Kittredge et al., 1967; Shelburne and Quin, 1967; Kirkpatrick and Bishop, 1973).

Ceramide-AEP (Hori et al., 1966; Higashi and Hori, 1968; Komai et al ., 1973) and the N-methyl-AEP-ceramide (Hori et al., 1969; Hayashi et al., 1969; Hayashi and Matsuura, 1971; Matsuura et al., 1973) have been isolated from fresh water molluscs. AEP in the free form, bound to lipid, and bound to protein has been found in terrestrial molluscs (Liang and Rosenberg, 1968). AEP has also been found in an echinoderm (Quin, 1965), a salt water crab (de Koning, 1970), the abalone (de Koning, 1966), the amoeba Acanthamoeba castellanii (Korn et al., 1973), mycobacteria (Sarma et al., 1970), Bdellovibrio bacteriovorus (Steiner et al., 1973), and the fungus Pythium prolatum (Wassef and Hendrix, 1977). Preliminary ^{31}P NMR studies with extracts from the clams Macrocallista nimbosa and Dinocardium robustum have indicated the presence of phosphonates (C.T. Burt and T.C. Myers, unpublished data). 1-Hydroxy-2-aminoethylphosphonic acid has been found bound to protein in an amoeba (Korn et al., 1973; Korn et al., 1974). More recently, the first example of a natural aliphatic β-unsaturated α-aminophosphonocarboxylic acid has been discovered in Streptomyces plumbens nov. sp. and identified as 2-amino-5-phosphono-3-pentenoic acid (Park et al., 1976).

Alkylphosphonic acids were not found in mammals until 1965, when AEP was isolated from bovine brain (Shimizu et al., 1965) and goat liver (Kandatsu and Horiguchi, 1965). Since then, AEP has been isolated from the nonpolar lipid and proteinaceous residue fractions of human brain (Alhadeff and Daves, 1970), the polar lipid and proteinaceous residue fractions of human liver, and from human heart and skeletal muscle (Alhadeff and Daves, 1971). N,N,N-Trimethyl-2-aminoethylphosphonic acid (cholinephosphonic acid) has also been

isolated from atherosclerotic plaques of human aorta (Alam and Bishop, 1968). Various lipid forms of methylated and unmethylated AEP have been isolated from bovine gall bladder bile (Tamari et al., 1976b; Tamari et al., 1976c) and bovine liver (Hasegawa et al., 1976a; Hasegawa et al., 1976c), including the novel conjugated bile acid ciliatocholic acid (Tamari et al., 1976c).

III. Metabolism

A. Metabolism of the C-P Covalent Linkage

1. Synthesis

^{32}P-Orthophosphate was found to be incorporated into AEP in T. pyriformis (Horiguchi, 1972a; Rosenberg, 1964), the fresh water mussel Hyriopsis schlegelii (Itasaka et al., 1969), and in marine phytoplankton (Kittredge et al., 1969). Because of this evidence that the carbon to phosphorus bond could be formed biosynthetically, efforts were begun to determine the pathways of phosphonate anabolism.

T. pyriformis incorporated ^{32}P-orthophosphate into 2-amino-3-phosphonopropionic acid (phosphonalanine; P-Ala) (Kittredge and Hughes, 1964). It was postulated that P-Ala could be the precursor of AEP by decarboxylation in a manner analogous to the formation of phosphatidylethanolamine from phosphatidylserine (Borkenhagen et al., 1961). It was subsequently found that P-Ala could act as a precursor of AEP, since approximately 19% of the ^{14}C-P-Ala taken up by T. pyriformis was found as AEP in the phosphonolipid (Smith and Law, 1970b) and ^{32}P-P-Ala was converted to AEP in broken cell preparations (Warren, 1968).

Through in vivo experiments with T. pyriformis utilizing radioactive precursors, Warren found that a glycolytic intermediate, most

likely phosphoenolpyruvate (PEP), was a precursor of AEP (Warren, 1968). These results were supported by the observations of others (Horiguchi et al., 1968; Liang and Rosenberg, 1968; Trebst and Geike, 1967). It was also found that P-Ala depressed the incorporation of ^{14}C-PEP into AEP in in vitro experiments with T. pyriformis (Horiguchi, 1972a), strengthening the hypothesis that P-Ala is a precursor of AEP. A recent proposed mechanism for the biosynthesis of AEP includes the rearrangement of PEP and then transamination to P-Ala, followed by deamination to 3-phosphonopyruvic acid and subsequent decarboxylation to 2-phosphonoacetaldehyde (Figure 2) (Horiguchi and Rosenberg, 1975). An efficient incorporation of 2-phosphonoacetaldehyde into AEP has been reported using cell-free preparations of T. pyriformis (Horiguchi, 1973).

- Rosenberg, in studying the growth of T. pyriformis with ^{32}P-orthophosphate, found the most rapid incorporation of label into lipid-bound AEP, and the slowest into the free AEP (Rosenberg, 1964). This is consistent with the involvement of a phosphatidyl derivative in the synthesis of AEP. The labelled fatty acids of choline phospholipids were later detected in 1,2-diacylglyceryl aminoethylphosphonate (diacylglyceryl-AEP), and Thompson postulated that phosphatidylcholine was a participant in the formation of a carbon to phosphorus bond (Thompson, 1969) (Figure 3). However, since no P-Ala was detected at the phospholipid level in T. pyriformis (Smith and Law, 1970a; Smith and Law, 1970b), the participation of a phospholipid derivative in the formation of AEP has not been proven.

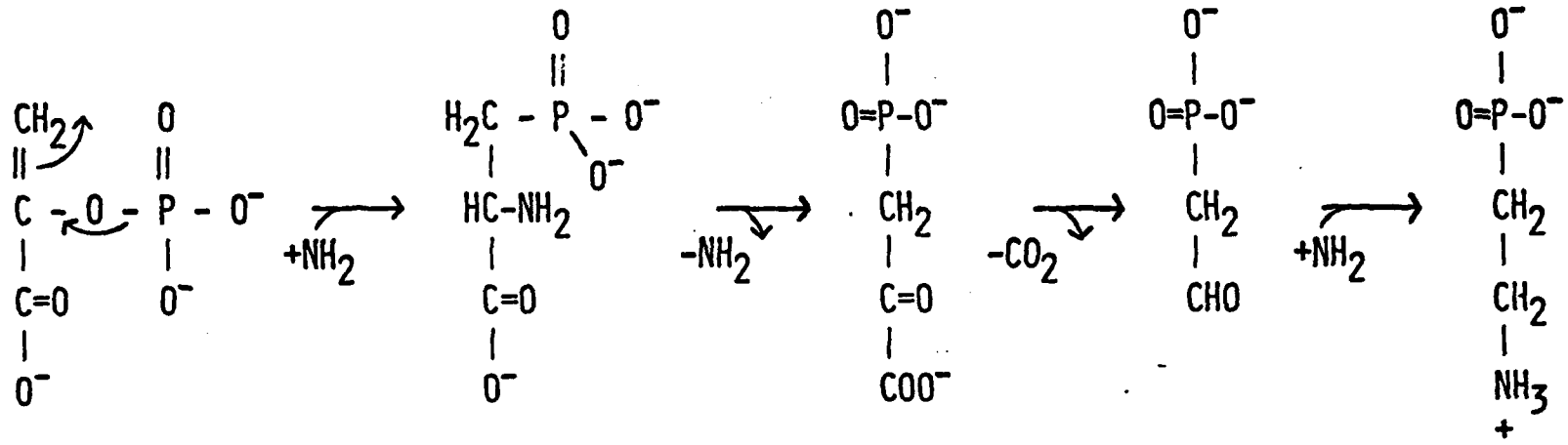

CH2
C
O
C=O
O⁻
O
P
O⁻
O⁻
+NH2
O
H2C - P - O⁻
O⁻
HC-NH2
C=O
O⁻
-NH2
O⁻
O=P-O⁻
CH2
C=O
COO⁻
-CO2
O⁻
O=P-O⁻
CH2
CHO
+NH2
O⁻
O=P-O⁻
CH2
CH2
NH3
+

FIGURE (2)

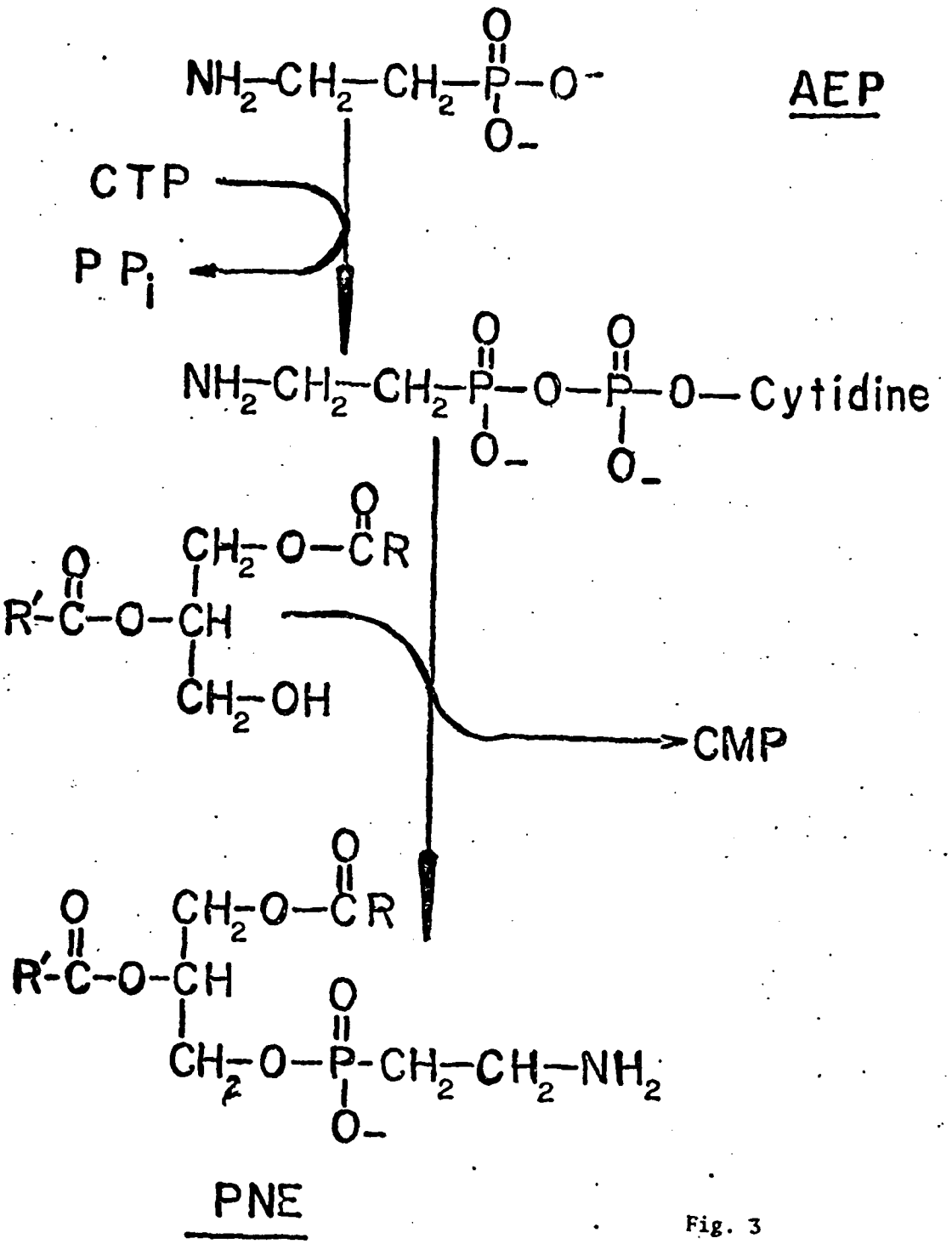


Fig. 3

In contrast to the above organisms, there appears to be no synthesis of AEP by mammals. *In vitro* experiments with rat liver slices utilizing U-^{14}C-glucose, 3-^{14}C-pyruvate and ^{32}P-orthophosphate and *in vivo* (rat) experiments using U-^{14}C-glucose demonstrated that no detectable biosynthesis of AEP from radioactive precursors occurred (Alhadeff *et al.*, 1972).

2. Degradation

A large number of bacterial species have been found to utilize alkylphosphonic acids as their sole source of phosphorus (Zeleznick, *et al.*, 1960; Mastalerz *et al.*, 1965; Harkness, 1966). One organism, *Bacillus cereus*, shows diauxie when grown with orthophosphate and AEP, with the orthophosphate being used first (Rosenberg and La Nauze, 1967). The degradation of AEP by *B. Cereus* was found to involve a transamination reaction (La Nauze and Rosenberg, 1967), and 2-phosphonoacetaldehyde was isolated as an intermediate in the degradation (La Nauze and Rosenberg, 1968). An enzyme "phosphonatase" has been isolated, which cleaves the carbon to phosphorus bond of phosphonoacetaldehyde (La Nauze *et al.*, 1970). An aldolase-like imine formation has been postulated in the enzyme's mechanism, which would labilize the C-P bond much in the same way that aldolase systems labilize C-C and C-H bonds (La Nauze *et al.*, 1977). This ability to cleave a direct carbon to phosphorus bond has not been demonstrated conclusively in any other organism (Tamari *et al.*, 1976a), although it can be presumed that other bacteria with the ability to grow on alkylphosphonic acids as their sole source of phosphorus do possess the ability to cleave the direct carbon to

phosphorus bond. In Pseudomonas aeruginosa it has been postulated that phosphonates are metabolized strictly through the cleavage of the C-P bond, without any modification of the amino groups on the phosphonates (Cassaigne et al., 1976).

B. Transport and Incorporation of Phosphonates

1. Protozoa

It has been determined that AEP is incorporated by T. pyriformis into diacylglyceryl-AEP, the phosphonate analogue of phosphatidylethanolamine (Sugita and Hori, 1971). In vivo and in vitro experiments have demonstrated the presence of CMP-AEP in T. pyriformis. In vitro experiments have shown that chemically synthesized CMP-AEP can transfer the AEP moiety to a diglyceride to form diacylglyceryl-AEP (Liang and Rosenberg, 1966). No methylation of diacylglyceryl-AEP occurred in T. pyriformis (Smith and Law, 1970a; Smith and Law, 1970b).

In P. aeruginosa, AEP transport is self-inducible, energy dependent, and competitive with inorganic phosphate and methylphosphonate (Lacoste et al., 1976). A second transport system for 3-aminopropylphosphonate (APP) has been found to be noncompetitively inhibited by phosphate and methylphosphonate. While AEP can be used as both a phosphorus source and a nitrogen source by the bacterium, APP is only used as a phosphorus source. In Escherichia coli 3, 4-dihydroxybutyl-1-phosphonate is similar to and competitive with glycerol-3-phosphate in its ability to inhibit cell growth (Shopsis et al., 1972). The phosphonate differs from glycerol-3-phosphate in that its inhibitory effect is maintained in the presence of glucose and inorganic phosphate.

2. Vertebrates

Since AEP and other alkylphosphonic acids have been isolated from lower organisms, it is likely significant quantities of phosphonates may be taken up by higher animals. In fact, 20-30 mg of AEP may be ingested upon eating eight mussels or four clams (Quin, 1967). The presence of AEP in bovine (Shimizu et al., 1965) and goat tissue (Kandatsu and Horiguchi, 1965), probably arising from the metabolism of rumen protozoa, indicates another source of phosphonates for human ingestion. Since it is likely that alkylphosphonic acids are ingested, the incorporation of AEP into mammalian tissues is of interest.

It was determined that ^{32}P-AEP was incorporated by the rat into at least two liver lipids and into lipid-free residues after 24 hours (Kandatsu et al., 1965). Upon oral administration of ^{32}P-AEP into rats, approximately 60% of the dose was absorbed from the intestine, with eventually 24% of the dose being accumulated in the body (Tamari et al., 1971). Later studies with CMP-^{14}C-AEP and ^{14}C-AEP (Tamari et al., 1975a; Hasegawa et al., 1976b) suggest that AEP taken into the liver is incorporated via a CMP-AEP intermediate into bile lipids and bile acids, possibly being methylated. Curley and Henderson found that 16% of the ^{14}C-AEP administered was incorporated into liver lipids as diacylglyceryl-AEP, which is the phosphonate analogue of phosphatidylethanolamine. Lipids from kidneys, heart, skeletal muscle, adipose, pancreas, and brain tissue contained less than 2% of the injected radioactivity (Curley and Henderson, 1972).

More recently, it was determined that the point of maximum incorporation of ^{3}H-AEP into liver lipids occurred from 12 to 30 hours after administration, compared to 2 to 3 hours for phosphorylethanolamine. The AEP was incorporated to the greatest extent into diacylglyceryl-AEP with some radioactivity co-chromatographing with the lyso-derivative. Maximum incorporation of ^{3}H-AEP into rat liver subcellular fractions took place in the soluble fraction. Maximum incorporation of radioactivity into liver lipids was seen in the microsomal fraction, with the next highest amounts in the nuclear and mitochondrial fractions (Curley-Joseph and Henderson, 1977). ^{32}P-AEP has also been found to be incorporated into lipids of rat tissues. The radioactive material was maximum in the liver lipids, with 89.3% of the incorporated radioactivity recovered in diacylglyceryl-AEP. These studies indicated that the incorporation of ^{32}P-AEP into lipids of liver subcellular fractions is maximum in the nuclear fraction and similar for mitochondria and microsomes. It was demonstrated that AEP was incorporated into three lipids of the rat liver (Maget-Dana et al., 1974).

In none of the earlier studies was there any evidence present for the cleavage of the carbon to phosphorus bond nor was there any methylation of the diacylglyceryl-AEP to phosphonolecithin. However, phosphonolecithin has been detected in the phospholipid fractions from bovine liver (Hasegawa et al., 1976a) and bile (Tamari et al., 1976b).

N-Trimethyl-2-aminoethylphosphonic acid (cholinephosphonic acid) was found to be incorporated *in vivo* into the phosphonate analogue of phosphatidylcholine, phosphonolecithin. No cleavage of the carbon to phosphorus bond was seen, nor was there any demethylation of phosphonolecithin (Bjerve, 1972). It was determined by an *in vitro* assay that AEP, while not being incorporated into brain lipids, hinders the utilization of ^{32}P-orthophosphate in the synthesis of phosphatidic acid, phosphatidylethanolamine and phosphatidylserine in brain slices, while higher concentrations decrease the synthesis of phosphatidylserine (Dana and Douste-Blazy, 1970).

Observations have been reported which suggest that the mechanism by which phosphonate compounds are incorporated into lipids is very likely to resemble the mechanism of incorporation of ethanolamine and choline into lipids (Kennedy and Weiss, 1956). Bjerve has shown that the incorporation of cholinephosphonic acid into phosphonolipids, is dependent upon the addition of CTP, and that this incorporation is inhibited by the addition of CDP-choline, indicating that CMP-cholinephosphonic acid is perhaps an intermediate in the synthesis of phosphonolecithin. It has been determined that cholinephosphonic acid acts as a competitive inhibitor in the conversion of phosphorylcholine and CTP to CDP-choline, and that phosphorylcholine acts as a competitive inhibitor when cholinephosphonic acid is the substrate for cytidyltransferase (Bjerve, 1972). From these data, it appears that the same enzyme which utilizes phosphorylcholine uses its phosphonate analogue in the synthesis of a CMP compound. In a similar study, AEP acted as a substrate for ethanolaminephosphate-cytidyltransferase, and also as a competitive inhibitor to phosphoryl-

ethanolamine (Plantavid et al., 1975). Very recently, data were published which indicate that ^{32}P-AEP and ^{14}C-AEP are incorporated in vitro and in vivo into rat liver lipids by a CMP intermediate (Tamari et al., 1973; Tamari et al., 1975a).

C. Phosphonoacetic Acid and Other Phosphonate Analogs

Phosphonoacetic acid (PAA) was first reported as an antiviral agent when it was found to inhibit replication of herpes simplex virus types 1 and 2 (Shipkowitz et al., 1973). Preliminary results indicated that PAA was an inhibitor of viral induced DNA polymerase but not normal cell DNA polymerase, and that the inhibition was not a result of any interaction with template DNA (Mao et al., 1975). Later, PAA was found to bind to the polymerase at the pyrophosphate binding site and act as a competitive inhibitor of pyrophosphate in an exchange reaction. An actual covalent linkage of deoxyribonucleoside 5'-monophosphate to PAA by a phosphodiester bond is postulated to account for the inhibition. PAA was also found to inhibit replication of the herpes virus of turkeys and of Marek's disease herpes virus (malignant lymphoma of chicken) (Leinbach et al., 1976). Honess and Watson isolated PAA resistant strains of herpes simplex virus-Type I but did not find PAA dependent clones. In addition, they found that mutants with different degrees of resistance to high concentrations of PAA may require multiple mutations (Honess and Watson, 1977). PAA has been found to inhibit α DNA polymerase from human cells (Bolden et al., 1975).

PAA has also been found to exhibit Epstein-Barr Virus DNA (EBV-DNA) replication in superinfected Raji Cells (Yajima et al., 1976), to inhibit EBV-DNA synthesis in vitro (Seebeck et al., 1977), and to inhibit transformation of human lymphocytes by EBV (Thorley-Lawson and Strominger, 1976).

A phosphonate antibiotic, phosphonomycin, which is effective against gram positive and negative microorganisms has been isolated from Streptomyces sp. (Hendlin et al., 1969). Two diphosphonate compounds (dichloromethylenediphosphonate and methylenediphosphonate) have been found to retard the rate of dissolution of apatite crystals in vitro, to inhibit bone resorption in tissue culture and in vivo, (Fleisch et al., 1969) and to inhibit pathological calcification in vivo (Francis et al., 1969).

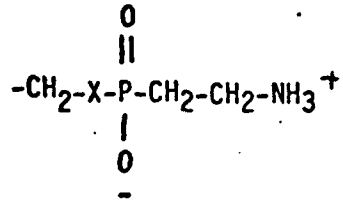
The glutamate analog 2-amino-4-phosphonobutyric acid has been shown to antagonize the excitatory action of glutamate on proteolipid receptors present in the intrathoracic muscle of the locust Schistocerca gregaria (Cull-Dandy et al., 1976).

Another group of phosphonate containing molecules which has greatly affected the metabolic field is the nucleotide analogs. These compounds do not undergo the usual phosphorylation and trans-phosphorylation reactions and are useful in inhibition studies involved with enzyme mechanisms and enzyme regulation. Much work has been done in this area, especially since the advent of ^{31}P NMR. However, this area will not be covered due to the nature of this review.

IV. AEP Associated with Proteinaceous Residues

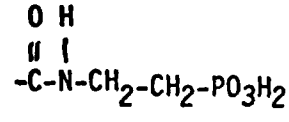
Quin found that a delipidated residue of the anemone M. dianthus contained 1.1% AEP by dry weight. Evidence for the occurrence of AEP in proteinaceous material was obtained by solubilization of the residue in 6 N HCl and precipitation of the solubilized protein using trichloroacetic acid (TCA). AEP was subsequently identified in the TCA precipitate (Quin, 1964). Quin proposed three general ways in which AEP could be bound into a polypeptide:

1) as a phosphonate monoester or amidate:

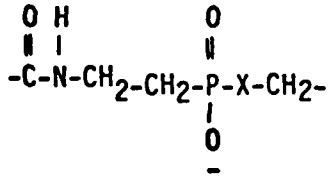


(X=NH or O)

2) as an amide:



3) as both 1 and 2:



(X=NH or O)

However, he found that the AEP bound in the residue did not form a DNP derivative and concluded that the residual AEP must be bound through either an amide or amide and monoester linkage (Quin, 1965).

Rosenberg found AEP in a similar insoluble residue of T. pyriformis. Following proteolysis of the residue with pronase and trypsin, the bulk of the AEP remained bound in unhydrolyzed material. From this data and data from incorporation of label from ^{32}P-orthophosphate, he concluded that AEP was incorporated into a macromolecular material, probably structural in nature (Rosenberg, 1964).

Peptidic and globular protein materials containing AEP and P-Ala were isolated from M. senile by Kirkpatrick and Bishop (Kirkpatrick and Bishop, 1971), who have more recently isolated a glycopeptide (molecular weight, 1500 daltons) from proteolytic digests of the same organism (Kirkpatrick and Bishop, 1972).

A phosphonate-rich glycoprotein containing 11% AEP, 22% neutral sugars, 4% hexosamines and 40% protein has ben prepared from M. dianthus (Hilderbrand et al., 1973). Amino acid analysis showed the presence of high relative amounts of aspartic acid, threonine, serine, glutamic acid, glucosamine, and galactosamine in addition to AEP (28% of the total ninhydrin reactive material).

Bunde et al. have since isolated two classes of proteins from M. senile. One class has a molecular weight greater than 10^7, 250 residues AEP/1000 amino acid residues and is 30% carbohydrate. The second class has a molecular weight less than 5×10^4, 50 residues AEP/1000 amino acid residue, and 7% carbohydrate. They propose that AEP is linked to N-acetylglucosamine which in turn is bonded to protein by O-seryl or O-threonyl glycosidic or N-aminoglycosidic linkage (Bunde et al., 1976). Kirkpatrick and Bishop have isolated a phosphonoprotein from Anthopleura xanthogrammica containing AEP (.03 μmole/mg) and N-methyl-2-AEP (.37 μmole/mg). They also determined that the distribu-

tion of AEP in M. senile was non-random, suggesting specific physiological functions for phosphonoproteins (Kirkpatrick and Bishop, 1973). Korn et al. have reported the occurrence of AEP and a novel aminophosphonic acid, 1-hydroxy-2-aminoethylphosphonic acid, in isolated amoeba plasma membrane. The macromolecular material was identified as a phosphonoglycan and is made up of about 13% phosphonates, 30% carbohydrates, and 6% amino sugars. The phosphonates were identified by amino acid analysis, mass spectrometry, and phosphorus and proton nuclear magnetic resonance (Korn et al., 1973; Korn et al., 1974).

V. The Role of Phosphonates in Living Organisms

As more invertebrates are being reinvestigated biochemically, the occurrence of AEP is more frequent than would be expected for a novel alkali stabile phosphorus containing amino acid. The widespread appearance of aminoalkylphosphonates in protozoans and lower metazoans gives reason to believe that biological phosphonates have an integral and necessary function in the animal kingdom. The majority of phosphonates have been found in variations of lipid structures such as ceramide-AEP or diacylglyceryl-AEP. Phosphonate enriched fractions have been isolated from membrane preparations of protozoans, and lipid extracts of coelenterates, echinoderms, and molluscs. The actual functional reason for phosphonolipids as opposed to phospholipids has not been determined. However, in starved oysters (Crassostrea virginica), phosphonolipids were conserved at the expense of phospholipids (Swift, 1977). So it may be that phosphonolipids are essential for membrane structure in the oyster, as could be true for other sea animals.

Mammalian metabolism studies on the incorporation of radioactively labeled AEP have shown a microsomal location for the incorporation activity. The actual enzymes involved in this process have not been isolated, nor have the N-methylation enzymes been identified yet. The mechanism for incorporation into phosphonolipids remains to be elucidated more completely. Since AEP is able to be modified in vertebrates, can one say there is no further metabolism of the molecule? The answer to the question will only come as newer studies and methods of detection (such as ^{31}P NMR) are employed in the area of mammalian phosphonate metabolism.

Human phosphonate research has been confined to isolation of different phosphonates from body organs, usually being a "one time" experiment. More thorough research is needed in the study of phosphonate forms present in the different primate species.

The function of the protein bound phosphonates present in sea anemones has still not been determined, nor have the actual sequences around the phosphonate area been determined. The phosphonoproteins have been shown to be extremely resistant to enzymatic hydrolysis, but seem to be quite labile to acid hydrolysis (freeing the amino end of the AEP molecule). Other researchers have noticed the acid lability, indicating a possible N- or O-glycosidic linkage being broken. The large amounts of AEP present in the phosphonoglycoproteins of Metridium would seem to indicate some sort of phosphonate polymer, as has been postulated by others (Kirkpatrick and Bishop, 1973). The possibility exists that the phosphonoglycoproteins are located in the mesogleal tissue, having structural properties, while not being an integral part of the collagen present.

SUMMARY

A literature review is made concerning the distribution, metabolism, and structural properties of naturally occurring alkylphosphonic acids (molecules which contain a covalent carbon-phosphorus bond). Alkylphosphonic acids, predominately as 2-aminoethylphosphonic acid (AEP), have been identified from a variety of specimens including bacteria, amoeba, protozoa, marine invertebrates, terrestrial molluscs, and from bovine, goat, and human tissue. The alkylphosphonic acids are found free and incorporated in lipid and protein materials. Miscellaneous other alkylphosphonic acids, such as the antiviral agent phosphonoacetic acid, may become useful in the future as therapeutic agents.

The metabolic information available from bacterial studies demonstrates that the C-P linkage can be anabolized and catabolized by enzymatic means. Although the exact mechanism is not known, recently proposed mechanisms are presented. The phosphonolipids are well defined structurally; however, the phosphonoproteins are not defined structurally and much remains to be determined concerning the cellular localization of the proteins and lipids. A function has yet to be determined for either the phosphonolipids or the phosphonoproteins although the broad distribution and unique character of the carbon-phosphorous bond indicates that phosphonates do serve or have served a useful biological purpose.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A literature review is made concerning the distribution, metabolism, and structural properties of naturally occurring alkylphosphonic acids (molecules which contain a covalent carbon-phosphorus bond). Alkylphosphonic acids, predominately as 2-aminoethylphosphonic acid (AEP), have been identified from a variety of specimens including bacteria, amoeba, protozoa, marine invertebrates, terrestrial molluscs, and from bovine, goat, and

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human tissue. The alkylphosphonic acids are found free and incorporated in lipid and protein materials. Miscellaneous other alkylphosphonic acids, such as the antiviral agent phosphonoacetic acid, may become useful in the future as therapeutic agents.

The metabolic information available from bacterial studies demonstrates that the C-P linkage can be anabolized and catabolized by enzymatic means. Although the exact mechanism is not known, recently proposed mechanisms are presented. The phosphonolipids are well defined structurally; however, the phosphonoproteins are not defined structurally and much remains to be determined concerning the cellular localization of the proteins and lipids. A function has yet to be determined for either the phosphonolipids or the phosphonoproteins although the broad distribution and unique character of the carbon-phosphorous bond indicates that phosphonates do serve or have served a useful biological purpose.


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8